

triplex, as the case may be). One of ordinary skill in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

5 Oligonucleotides that are complementary to the 5' end of the message, for example, the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated

10 sequences of mRNAs recently have been shown to be effective at inhibiting translation of mRNAs as well (Wagner, *Nature* 372:333, 1984). Thus, oligonucleotides complementary to either the 5' or 3' non-translated, non-coding regions of a GLUTX gene, could be used in an antisense approach to
15 inhibit translation of endogenous GLUTX- mRNA.

Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon.

Antisense oligonucleotides complementary to mRNA
20 coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5', 3', or coding region of GLUTX mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides
25 ranging from 6 to about 50 nucleotides in length. In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides, or at least 50 nucleotides.

Regardless of the choice of target sequence, as with
30 other therapeutic strategies directed to GLUTX, it is preferred that *in vitro* studies are first performed to assess the ability of an antisense oligonucleotide to inhibit gene expression. If desired, the assessment can be

quantitative. It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and any nonspecific biological effect that an oligonucleotide may cause. It is also preferred that these studies compare levels of the target RNA or protein with that of an internal control RNA or protein. Additionally, it is envisioned that results obtained using an antisense oligonucleotide are compared with those obtained using a control oligonucleotide. Preferably, the control oligonucleotide is of approximately the same length as the test oligonucleotide, and the nucleotide sequence of the control oligonucleotide differs from that of the test antisense sequence no more than is necessary to prevent specific hybridization between the control oligonucleotide and the targeted RNA sequence.

The oligonucleotides can contain DNA or RNA, or they can contain chimeric mixtures, derivatives, or modified versions thereof that are either single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. Modified sugar moieties can be selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose. A modified phosphate backbone can be selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal, or an analog of any of these backbones.

The oligonucleotide can include other appended groups such as peptides (e.g., for disrupting the transport properties of the molecule in host cells *in vivo*), or agents that facilitate transport across the cell membrane (as

described, for example, in Letsinger et al., *Proc. Natl. Acad. Sci. USA* 86:6553, 1989; Lemaitre et al., *Proc. Natl. Acad. Sci. USA* 84:648, 1987; PCT Publication No. WO 88/09810) or the blood-brain barrier (see, for example, PCT Publication No. WO 89/10134), or hybridization-triggered cleavage agents (see, for example, Krol et al., *BioTechniques* 6:958, 1988), or intercalating agents (see, for example, Zon, *Pharm. Res.* 5:539, 1988). To this end, the oligonucleotide can be conjugated to another molecule, for example, a peptide, a hybridization triggered cross-linking agent, a transport agent, or a hybridization-triggered cleavage agent.

An antisense oligonucleotide of the invention can comprise at least one modified base moiety that is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethyl-aminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 2-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

In yet another embodiment, the antisense